Studies on the Proteose-peptone Fraction of Milk: Isolation & Properties of Proteose-peptone & Proteose from Cow & Buffalo Milk

2425 **Some of the physical and chemical properties of proteose-peptone and proteose fractions, isolated from cow and buffalo milk in yields of 173 mg. and 155 mg./100 ml. milk respectively, have been investigated. Proteose-peptone from cow and buffalo milk contains the same 17 amino acids, but quantitatively there is no similarity in their amino acid make-up. Paper electrophoretic resolution of proteose from cow milk shows three components in it whereas proteose from buffalo milk shows only two components; proteose components from buffalo milk show greater electrophoretic mobility than the proteose components of cow milk. Cystine and serine are the two N-terminal amino acids of both proteose and proteose-peptone from cow and buffalo milk. The proteose-peptone content of cow, buffalo and goat milk varies, cow milk containing the highest amount and goat milk the least. Colostrum from cow and buffalo has a greater proteose-peptone content than milk.**

PROTEOSE-PEPTONE, a minor component of milk and the elucidation of its role during processing of milk for the preparation of products, is of interest. Limited information is available on the physical and chemical properties of proteose-peptone in milk. This fraction, as defined by Rowland[1], is present in milk whey obtained by heating milk to 95°C. for 30 min. and precipitating the milk solids by 10 per cent trichloroacetic acid (TCA). Chemical analysis of proteose-peptone fraction of cow milk has been reported by Brunner and Thompson[2]. The amino acid composition of proteose-peptone from cow milk has been investigated by Weinstein *et al.*[3] and its electrophoretic behaviour by Aschaffenburg[4], and Larson and Rolleri[5]. Brunner and Thompson[6] have reported the characteristics of four minor protein fractions, namely the proteose-peptone of Rowland[1], the σ-proteose of Aschaffenburg[4], the minor protein fraction of Weinstein *et al.*[3] and the milk component '5' of Jenness[7] isolated from the same milk sample.

The present study was undertaken to collect data on certain chemical and physical properties of the proteose-peptone (TCA precipitable components) and proteose (ammonium sulphate precipitable fraction) isolated from the same samples of cow and buffalo milk. The amino acid composition of proteose-peptone fraction from the two sources has been determined and compared with that of casein from cow and buffalo milk. Electrophoretic mobilities of the components of proteose from cow and buffalo milk have been investigated. The R_f values of dinitrophenyl (DNP)-amino acids and the N-terminal amino acids present in proteose and proteose-peptone from cow and buffalo milk have been determined. The results of these studies are reported in this paper and compared with the results obtained by other workers.

Experimental Procedure

Isolation of proteose and proteose-peptone — Proteose was isolated from milk according to Aschaffenburg[4] with suitable modifications. Proteose-peptone was isolated according to Rowland[1] as follows. Raw milk (500 ml.) was diluted 4 times with distilled water, heated to 40°C., treated with acetic acid and sodium acetate to precipitate casein, and filtered through Whatman No. 42 filter paper[3]. Whey proteins were removed from the filtrate by heat denaturation[1,3]. The filtrate thus obtained was divided into two equal halves. To one part, 35 g. of solid ammonium sulphate per 100 ml. filtrate were added to precipitate 'proteose' and from the other half 'proteose-peptone' was precipitated by adding 8 g. of TCA per 100 ml. of filtrate. The precipitates were kept overnight at 4-5°C. and collected by centrifugation at 10000 *g* at room temperature. The proteose and proteose-peptone precipitates thus obtained were separately dispersed in 20-30 ml. of distilled water and dialysed first against tap water for two days which rendered the precipitate soluble and subsequently they were dialysed against distilled water for another day at 4-5°C. At this stage, insoluble material, if any, was removed by centrifugation. The dialysed solutions were finally dried *in vacuo* over NaOH and weighed.

Amino acid analysis — Dry sample of proteose-peptone (20 mg.) was hydrolysed with 2 ml. of 5·7*N* HCl for 16 hr in a sealed tube at 110°C. The hydrolysate was freed from acid by drying *in vacuo* over NaOH. The dried residue was dissolved in 2 ml. of distilled water and analysed for amino acids by paper chromatography.

The amino acid composition of the acid hydrolysate was determined quantitatively employing two-dimensional paper chromatography with phenol-water and *n*-butanol-acetic acid-water (4:1:1) as solvents according to the procedure of Ghosh Mazumder *et al.*[8]. Final identification of the amino acids was carried out using specific reagents[9]. For comparing the amino acid make-up of proteose-peptone samples from cow and buffalo milk, circular paper chromatographic technique of Ganguli[10] was also used.

Paper electrophoresis of proteose samples — Proteose samples were subjected to paper electrophoresis according to the method of Ganguli and Bhalerao[11]. A 2 per cent solution (5-10 μl.) of the sample in water was applied on the paper disk and a current of 10-15 ma. and 400 V. was applied for 3-4 hr to achieve good resolution of the components. For a comparative study, solutions of proteose samples from cow and buffalo milk were applied on the same paper. Electrophoretic mobilities of the components of the proteose samples were studied at different *p*H values employing buffers whose *p*H values ranged from 4·5 to 10·0.

N-Terminal amino acid analysis — The N-terminal amino acids present in proteose and proteose-peptone were detected separately by the fluorodinitrobenzene (FDNB) procedure of Sanger[12]. The separation of the dinitrophenyl (DNP)-proteose, and DNP-proteose-peptone, hydrolysis of the derivatives and subsequent identification of the amino acids were done according to the methods of Fraenkel-Conrat *et al.*[13] and Biserte *et al.*[14] respectively. The details of the analytical procedure followed for the final detection of the amino acids were the same as those described earlier by Ganguli *et al.*[15] for the N-terminal amino acid analysis of casein.

Other analyses — The proteose-peptone content of milk and colostrum was estimated by the colorimetric method of Ganguli *et al.*[16]. Tryptophan was estimated by the ultraviolet spectroscopic method of Beaven and Holiday[17] using a 5-10 mg./ml. solution in 0·1*N* NaOH and noting the absorbancy at 280·5 mμ in a Beckman spectrophotometer in cells of 1·0 cm. light path. An extinction coefficient of 5430 was used in calculation. Proline was determined by the isatin reagent[18].

Results and Discussion

The dry samples of proteose and proteose-peptone have a shiny appearance but are not crystalline. They are highly soluble in water, non-dialysable and ninhydrin positive, and exhibit surface active property. The proteose-peptone content of milk samples from Red Sindhi, Sahiwal and Tharparkar breed of cows and from Murrah buffalo, as determined by the isolation procedure employed, is recorded in Table 1 along with the values determined colorimetrically[16]. The yield of proteose was 100-150 mg./100 ml. milk as compared to 190-240 mg./100 ml. for proteose-peptone. These values for proteose-peptone, after correction for the losses during fractionation of milk, agree fairly well with the corresponding values for proteose-peptone estimated by the colorimetric method[16]. The quantity of proteose-peptone obtained was greater than that of proteose from the same milk sample.

Amino acid composition of proteose-peptone — The amino acid composition of proteose-peptone from cow and buffalo milk recorded in Table 2 shows that both the proteose-peptones contain the same 17 amino acids, but in quantitative terms the two proteose-peptone samples differ. The amounts of arginine, serine, glutamic acid, tyrosine, alanine, leucines, proline and tryptophan present in proteose-peptone samples from cow and buffalo milk are about the same but the amount of other amino acids differ. The proteose-peptone samples are lacking in valine which is present in casein[19] along with other amino acids. Quantitatively also, the composition of proteose-peptone is definitely different from that of casein.

TABLE 1 — PROTEOSE-PEPTONE CONTENTS OF COW AND BUFFALO MILK

(Ten samples of milk analysed in each case)

	Proteose-peptone mg./100 ml. milk	
	Isolation procedure	Estimated chemically
Sahiwal cow	140	160
Red Sindhi cow	180	195
Tharparkar cow	200	205
Murrah buffalo	155	160

TABLE 2 — AMINO ACID COMPOSITION OF PROTEOSE-PEPTONE ISOLATED FROM COW AND BUFFALO MILK AS DETERMINED BY PAPER CHROMATOGRAPHY

(Amino acid content expressed in g./100 g. of proteose-peptone)

Amino acid	Proteose-peptone*		Casein†	
	Cow	Buffalo	Cow	Buffalo
Arginine	2·37	2·37	4·71	2·78
Alanine	4·86	4·05	2·98	2·37
Aspartic acid	7·62	11·93	28·63‡	27·28‡
Glutamic acid	7·92	7·75		
Cysteine	5·00	6·96	—	—
Glycine	4·05	1·70	8·90	8·85§
Serine	3·10	3·45		
Histidine	6·00	7·30	1·38	1·62
Leucines	9·13	9·00	13·55	13·26
Lysine	6·42	7·42	8·47	7·56
Methionine	4·94	3·60	2·48	2·01
Phenylalanine	3·19	4·26	4·99	4·46
Proline	10·70	10·30	—	—
Threonine	3·50	2·90	4·31	3·74
Tryptophan	4·21	4·21	1·30	1·45
Tyrosine	2·96	2·45	4·80	4·21

*Average composition based on the analysis of 15 samples.
†Values reported by Ganguli, N. C., Prabhakaran, R. J. V. & Iya, K. K., *J. Dairy Sci.*, **47** (1964), 13.
‡Value for both aspartic acid and glutamic acid.
§Value for both glycine and serine.

Weinstein *et al.*[3] have analysed a similar proteose-peptone fraction and their results agree with those reported here. The minor differences observed may be due to the fact that the fraction analysed by Weinstein *et al.*[3] is not the same as that used in the present study. The minor protein fraction isolated by Weinstein *et al.*[3] contained the glycopeptide in addition to proteose-peptone because rennet was used for removing casein from milk instead of acid.

Electrophoretic behaviour of proteoses from cow and buffalo milk — A typical paper electrophorogram of proteose components from cow and buffalo milk is shown in Fig. 1 and their electrophoretic mobilities at different *p*H are given in Table 3. The results show that the isoelectric point for both samples of proteoses lies between *p*H 4·5 and 6·0. The proteose from

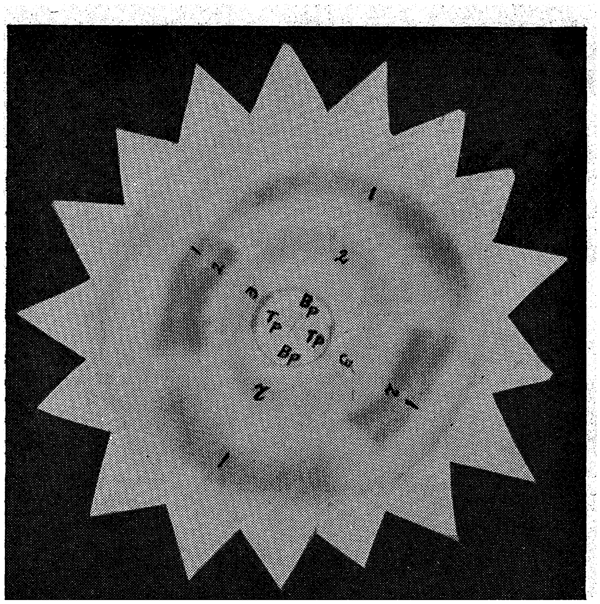

Fig. 1 — Paper disk electrophorogram of proteose samples from cow and buffalo milk [T_p, proteose from Tharparkar cow milk and B_p, proteose from buffalo milk; the numbers 1, 2 and 3 refer to the components of the proteoses]

TABLE 3 — ELECTROPHORETIC MOBILITIES OF PROTEOSE COMPONENTS FROM COW AND BUFFALO MILK

*p*H of buffer	Mobility, mm.		
	First band	Second band	Third band
	COW MILK		
4·5	0·00	0·00	0·00
5·0	0·00	0·00	0·00
6·0	3·00	0·00	0·00
7·0	23·00	20·00	0·00
9·0	43·00	32·00	12·00
10·0	55·00	39·00	18·00
	BUFFALO MILK		
4·5	0·00	0·00	0·00
5·0	0·00	0·00	0·00
6·0	18·00	0·00	0·00
7·0	23·00	20·00	0·00
9·0	47·00	39·00	0·00
10·0	62·00	47·00	0·00

buffalo milk shows greater mobility than the proteose from cow milk in the *p*H range between 6·0 and 10·0. The electrophorogram (Fig. 1) shows that cow milk proteose is made up of three components whereas buffalo milk proteose has only two components. The existence of two to three components in milk proteose which can be electrophoretically separated has been reported. Aschaffenburg[4], and Larson and Rolleri[5] detected three components in proteose isolated from cow milk whereas Weinstein *et al.*[3] detected only two components in their minor protein fraction. Brunner and Thompson[6] demonstrated the multicomponent nature of the minor protein fraction by the free-boundary electrophoretic method. These workers achieved best resolution of the proteose components at *p*H 7·0 in veronal-citrate buffer whereas in the present study satisfactory resolution was achieved only at *p*H 9·0 using veronal-HCl buffer. Thus the minor protein fraction in cow and buffalo milk significantly differs in its electrophoretic behaviour from that of casein, the major milk protein[20].

N-Terminal amino acid residues of proteose and proteose-peptone — The DNP derivatives of the amino acids, obtained after treatment of proteose and proteose-peptone with FDNB, were soluble in *n*-butanol. Ammonolysis[16] of the derivatives and paper chromatography revealed two ninhydrin positive spots on separation by *n*-butanol-acetic acid-water (4: 1: 1). The results summarized in Table 4 indicate that both proteose and proteose-peptone from cow and buffalo milk have the same two N-terminal amino acids, cystine and serine. Incidentally, it may be mentioned here that the detection of cystine as the N-terminal amino acid was only possible when experiments were carried out on a preparative scale. Proteose and proteose-peptone differ in their N-terminal amino acids from the major protein casein in which arginine and lysine are present at the N-terminal end[15]. If proteose or proteose-peptone is obtained from casein, the results of the present study show that they should appear as cleaved products from the C-terminal end.

Proteose-peptone content of milk and colostrum — The proteose-peptone contents of cow, buffalo and goat milk, estimated colorimetrically[16], are presented in Table 5. It is evident from the results that cow milk has the highest concentration of proteose-peptone (220 mg./100 ml.) followed by buffalo milk (172 mg./100 ml.) and goat milk (56 mg./100 ml.). The proteose-peptone content of cow milk reported in the present study agrees with

TABLE 4 — SOME CHARACTERISTICS OF THE N-TERMINAL AMINO ACID DERIVATIVES OBTAINED FROM PROTEOSE AND PROTEOSE-PEPTONE

Fraction analysed	Source of sample	R_f value of DNP-amino acids	N-Terminal amino acids
Proteose	Cow	0·42	Cystine, serine
Proteose-peptone	do	0·41	do
Proteose	Buffalo	0·40	do
Proteose-peptone	do	0·41	do

TABLE 5 — PROTEOSE-PEPTONE CONTENT OF COW, BUFFALO AND GOAT MILK

Source of milk	No. of samples analysed	Proteose-peptone mg./100 ml.	
		Range	Average
Cow	70	51-395	220
Buffalo	40	82-282	172
Goat	15	10-112	56

Table 6 — Concentration of Proteose-peptone in Colostrum from Cow and Buffalo

Post partum day	Proteose-peptone, mg./100 ml.	
	Cow	Buffalo
1	376*	302*
2	230	282
3	192	268
4	173	169
6	170	110

*Average proteose-peptone content determined from 10 samples of first day secretion was 306 mg. and 236 mg./100 ml. of cow and buffalo milk respectively.

that reported by Shahani and Sommer[21]. The variations reported for the proteose-peptone content of milk are probably due to lactation stage of the animal[22]. The low proteose-peptone content of goat milk is interesting and merits further study.

The average proteose-peptone content of colostrum collected on the first day after parturition is much higher compared to milk samples (Table 6). Colostrum from cow has a higher proteose-peptone content (306 mg./100 ml.) than colostrum from buffalo (236 mg./100 ml.).

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